

# The truffle-like *Protuberera canescens* is an early developmental stage of the Cage Fungus *Ileodictyon*

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## Abstract

Recent collections from a site in the Melbourne suburb of Altona North indicate that the truffle-like fungus *Protuberera canescens* is the young stage of the Cage Fungus *Ileodictyon gracile*. Over several months, fruit-bodies consistent with descriptions of *Protuberera canescens* were observed, but later, at the same site, lattices emerged, typical of *Ileodictyon gracile*. In the unopened fruit-bodies radial threads were observed in the gelatinous layer of the peridium. These structures match with literature reports of the morphology of both species, although they have been little discussed previously in *Ileodictyon gracile*. Two other truffle-like species, *Protuberera africana* and *Rhizopogon rodwayi*, have the radial threads and co-occur with *Ileodictyon gracile*. Thus, the possibility that they are also young stages of *Ileodictyon* should be explored. Development in *Ileodictyon* is unusual in combining the benefit of an enclosed, hypogeous truffle-like stage, where spore production is protected from desiccation, and an epigeal lattice-stage, from which spores are dispersed by insects. (*The Victorian Naturalist* 127 (2), 2010, 49–54)

**Keywords:** Phallales; Clathraceae; Protophallaceae; Lattice Fungus; hypogeous fungi

## Introduction

*Ileodictyon* is the familiar Cage or Lattice Fungus, comprising two species, *I. cibarium* and *I. gracile*, both widespread in Australia (Grey and Grey 2005). They emerge from an egg-like structure and form a lattice with a slimy spore mass on the inside. In contrast, *Protuberera* forms underground truffle-like fruit-bodies with the spore mass (gleba) remaining enclosed (indehiscent) within an outer layer (peridium). There are about a dozen species of *Protuberera*, one of which, *Protuberera canescens*, has been described from Australia (Beaton and Malajczuk 1986; Malloch 1989; Castellano and Beever 1994).

In classifications based on morphology, both *Protuberera* and *Ileodictyon* have been placed in the Phallales (the stinkhorn fungi), but the former genus has been considered to belong to the family Protophallaceae due to the indehiscent fruit-body, while the latter genus has been placed in the Clathraceae (Hawksworth *et al.* 1983) where the fruit-body emerges from an 'egg' to form an above ground fruit-body with arms, columns, tentacles or a lattice.

In a recent phylogenetic analysis based on several DNA regions, Hosaka *et al.* (2006) found that species previously assigned to *Protuberera* fell within five different families: Clathraceae (*Protuberera canescens*), Gallaceaceae (*P. nothofagi*), Lysuraceae (*P. clathroidea*), Phallogastraceae (*P.*

*hautuensis*) and Protophallaceae (several species including the type of the genus, *P. maracuja*). Hosaka *et al.* (2006) suggested that those *Protuberera* species that fell within families that otherwise contained stinkhorns (Lysuraceae) and lattice stinkhorns (Clathraceae) were likely to be unopened stinkhorn fruiting bodies. We report here field observations that confirm that *Protuberera canescens* is an early developmental 'egg' stage of *Ileodictyon*.

## Observations at Altona North

While looking for fungi along a stretch of Kororoit Creek on 21 May 2006, two of the authors (AS and NS) came across a large cluster of white, spherical fungal fruit-bodies, 3–8 cm diameter, on or half-buried in deep, rich, loose soil near or under native trees and shrubs (Fig. 1a). The location was at the bottom of a bank south-west of (and below) John Street, near Harris Reserve in Altona North.

When handled, the white spheres felt gelatinous inside, so they were not just young puffballs, and they had white rhizomorphs (root-like structures) at the base. Further examination, particularly of the cross section (Fig. 1b), revealed that they had a three-layered peridium. The outer layer was thin and membranous, and the inner layer (which bounded the greenish-olive gleba) was also very thin.

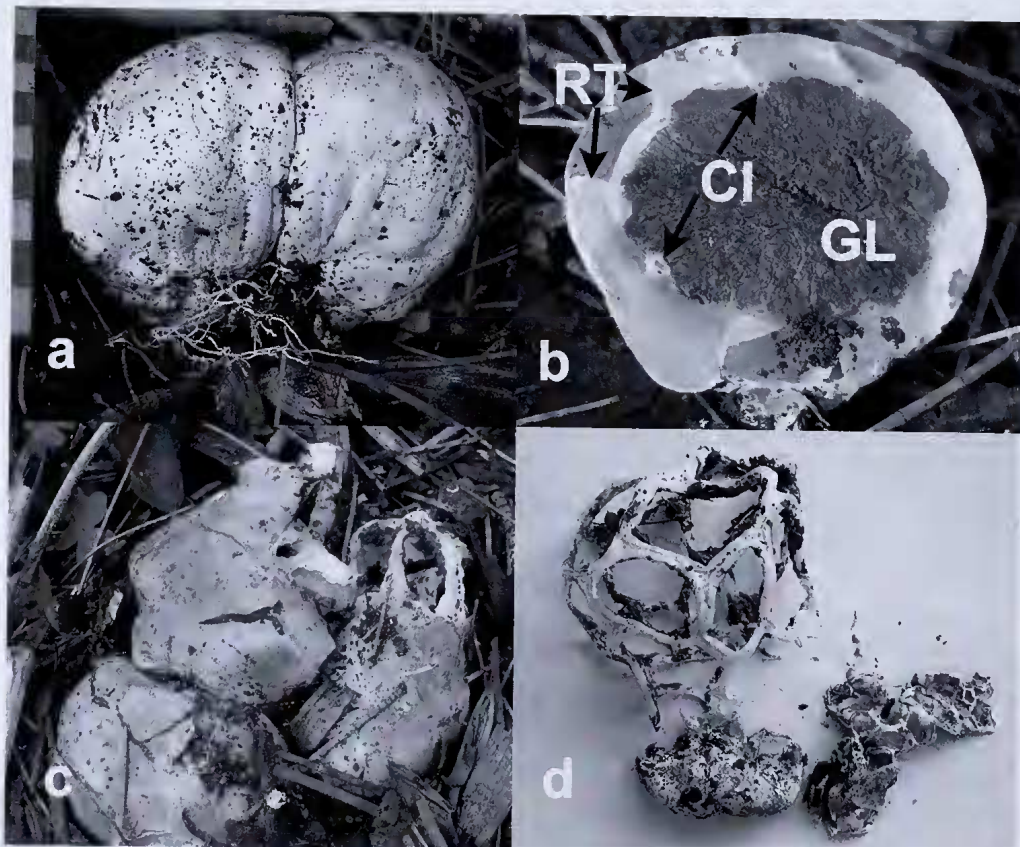


Fig. 1. *Ileodictyon gracile* from Altona North. a. unexpanded fruit-body (each segment on the scale at left is 1 cm); b. cross section of unexpanded fruit-body (agreeing with structure of *Protuberia canescens*), note the radial threads in the gelatinous layer of the peridium (RT) and the circular structures (CI) at the base of the threads, adjacent to the gleba (GL); c. partially expanded lattice arising from ruptured peridium; d. fully expanded lattice and remnants of peridium (lower right) showing polygonal pattern on inner surface. Photos: Alice Sinnott.

Between the inner and outer layers there was a thick gelatinous layer through which ran radial, white threads, dividing the middle layer into roughly equal compartments. This peridium structure matches closely that of *Protuberia canescens*, as described from Western Australia by Beaton and Malajczuk (1986) and illustrated by Bougher and Syme (1998: p. 127).

Fruit-bodies of *Protuberia canescens* and *Ileodictyon* had previously been observed (by TM) at roughly the same spot in the Royal Botanic Gardens Melbourne, although at quite different times. This raised the possibility that the former might be the immature, unopened 'egg' stage of the latter. In support of this hypothesis, when a fruit-body of the Altona North mate-

rial was gently cut so that a section of the outer peridium and gelatinous layer was peeled away, the whitish radial threads (sutures, see Fig. 1b, RT) revealed on the surface of the central gleba formed a mosaic or lattice pattern very reminiscent of an *Ileodictyon*. The arms of this lattice 'in miniature' can be seen in cross sections as small circular structures sitting at the base of the radial threads in the gelatinous layer, at the boundary between the gelatinous layer and the gleba (Fig. 1b, CI).

As the Altona North site was easy to get to, and there were numerous fruit-bodies, it was clear that an excellent opportunity had arisen to check whether the peridium of *P. canescens* really remained closed or not, and a watching

brief was kept on this location. Several further visits by NS and AS revealed most of the fruit-bodies unchanged, though a number had been disturbed and damaged. A likely explanation for this was that birds had dug out and pecked open fruit-bodies in search of insect larvae that were frequently observed feeding on the gleba.

On a visit on 25 June 2006 there were small, almost dried-up *Ileodictyon* lattices at the site. These had all been disturbed, and it was not clear whether the lattices were the result of natural opening or of damage by birds. Undisturbed fruit-bodies, of which there were still plenty, remained closed.

The situation became clearer on 1 July when some, at least, of the lattices were found to have emerged naturally (Figs 1c, 1d). In fact, one lattice, which had begun to emerge when the fruit-body was picked up, expanded in one of the collectors' hands while the other collector was photographing it. Most of the fruit-bodies, however, were still closed.

A number of unopened, undamaged fruit-bodies were removed and stored, half-covered with soil from the site, in a large pot. None of the potted fruit-bodies opened, and by 7 August all had disappeared, probably eaten away from the inside by the larvae that relished the gleba. A few more lattices appeared at the Altona North site in July, but most of the undamaged fruit-bodies remained unopened. On 20 August there were no longer any fruit-bodies at the site, just white mycelium under the soil and a few fragments of dried peridium. Below average rainfall in winter 2006 may have contributed to the lack of expansion of lattices.

#### Synonymy of *Protuberera canescens* with *Ileodictyon gracile*

*Protuberera canescens* was described as *Protuberera* because the Western Australian material was not seen to open and yet contained apparently mature spores. The only difference between the type description of *Protuberera canescens* and the Altona North material is that Beaton and Malajczuk (1986) do not explicitly note the presence in cross section of circular structures at the base of the radial threads (which they call 'sutures') traversing the gelatinous layer of the peridium. However, close examination of the original illustrations of *Protuberera canescens* (Beaton and Malajczuk 1986) shows a slight expansion of at least some of the threads where they meet the gleba. Field notes with a collection identified as

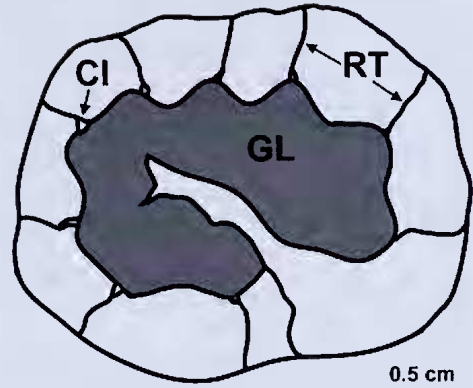


Fig. 2. Diagram of cross section of the '*Protuberera canescens*' form of *Ileodictyon gracile* from Belgrave South, showing radial threads (RT) bifurcating adjacent to the gleba (GL) to form circular or polygonal structures (CI).

*Protuberera canescens* from Belgrave South (Fig. 2) clearly depict the radial threads, with most of them bifurcating immediately adjacent to the gleba, to form triangular or irregularly circular structures. In the illustration in Beaton and Malajczuk (1986) there is also some bifurcation of the threads at the other end, immediately under the outer layer of the peridium, but we have not observed this in Victorian material.

Radial threads in the gelatinous layer at an early stage of fruit-body development have not been highlighted for *Ileodictyon*, but are a characteristic of the Clathraceae (Dring 1980). Radial threads are not mentioned in the descriptions of *I. cibarium* and *I. gracile* by Cunningham (1944), although in the accompanying figure of a cross section of an unopened *I. cibarium* egg (pl. XII, fig. 4), the radial threads are visible within the thick gelatinous layer of the peridium, and at their inner end are many structures with convoluted outlines, that are the developing arms of the lattice. Semmens (1967), in an overlooked description of the development of *Ileodictyon gracile* (as *Clathrus gracilis*), does mention the radial threads, calling them 'hyphal threads forming a series of membranous division-walls'. He also notes that the tubes that will eventually form the fruit-body are initially attached to the base of the radial threads, adjacent to the gleba, and he depicts the incipient tubes as roughly circular in cross-section (Semmens 1967, plate XIV-7), although he does not show the tubes as occur-



ring at the base of each radial thread. The radial threads are also illustrated by Dring (1980: fig. 17E) for *Ileodictyon gracile*, who does depict the developing lattice tubes as occurring at the base of each thread (as observed in the Altona North material). Castellano and Beever (1994) comment on the general similarity between *Protuberata* and the Clathraceae, by virtue of the presence of radial threads ('sutures') and small, smooth spores, but they do not comment further on the great similarity between *Ileodictyon* and species of *Protuberata* with a thick gelatinous middle layer to the peridium.

We did not fully investigate the three-dimensional structure of the radial threads, but Semmens (1967) shows that what appears as threads in cross section are thin plates that are embedded in the gelatinous middle layer of the peridium. When the lattice has fully developed, the loose and separated fragments of the peridium show a polygonal pattern on the inner surface (Fig. 1d and Grey and Grey 2005: 99). This pattern shows the edges of those thin plates that formed the arms of the lattice and indicates where they have separated from it. This polygonal pattern is also illustrated by 'Gaye from the Hunter' (2009).

The presence of radial threads in the thickened, gelatinous mid-layer of the peridium is remarkably similar in both *Protuberata canescens* and the unopened stages of *Ileodictyon*. In other respects, apart from the maturity of the lattice itself, *Protuberata canescens* and *Ileodictyon* are also very similar, including sharing abundant white rhizomorphs (although these are not unusual in the Phallales).

Observation of the development of the Altona North material from unexpanded fruit-bodies to those producing a lattice shows that the morphology of the young stage of an *Ileodictyon* matches that of the description for *Protuberata canescens*. This conforms to the finding of Hosaka *et al.* (2006) that collections identified as *Protuberata canescens*, *Ileodictyon gracile* and *I. cibarium* were all extremely closely related in a phylogenetic analysis of DNA sequence data.

If *Protuberata canescens* is an *Ileodictyon*, to which species does it belong? The two species of *Ileodictyon*, *I. gracile* and *I. cibarium*, are distinguished in the lattice stage by the former having sinuously folded arms, which are expanded where joined, and the latter having concertina-like folding of the arms, which do not expand

where joined (Cunningham 1944; Grey and Grey 2005). Lattices of the Altona North material fit well with the characteristics of *I. gracile*.

Hosaka *et al.* (2006) included three collections of *I. gracile* and one of *I. cibarium* in their analysis of molecular data. It is not clear from the phylogenetic tree in Hosaka *et al.* (2006) if collections of the two species fall in separate clades (monophyletic clusters), because there is very little difference in the DNA sequences among the collections of both species. The two collections of *Protuberata canescens* which were included do not cluster immediately adjacent to one another—one seems to fall with collections of *I. gracile*, with the other closer to the collection of *I. cibarium*. There is very little difference between sequence data for the *Protuberata canescens* collections and that of the two *Ileodictyon* species. Further analysis of morphology and DNA sequence data that includes multiple samples from both species of *Ileodictyon* is required to confirm that *I. gracile* and *I. cibarium* are independent species, and to establish if the unexpanded material of both species has the form of *Protuberata canescens*, or if there are subtle differences in the unexpanded stage of the two species of *Ileodictyon*.

Within other families of the Basidiomycota, regions such as the Internal Transcribed Spacer (ITS) of the ribosomal DNA have been found to vary slightly within species, but differ between most pairs of species, such as in *Cortinarius* (Frøslev *et al.* 2007). The ITS was not one of the regions included by Hosaka *et al.* (2006) and analysis of sequences from this region for *Ileodictyon* would be of interest.

Formal synonymy of *Protuberata canescens* with *Ileodictyon gracile* is not warranted until unexpanded fruit-bodies of *Ileodictyon cibarium* can be compared to those of *I. gracile* to determine if there are any distinguishing characters between the two species at an early stage of development.

#### Synonymy of other taxa with *Ileodictyon gracile*

In the type species of *Protuberata* (*P. maracuja* Möller) fruit-bodies remain closed in all stages of development from initiation to final dissolution (Möller 1895 as cited by Malloch 1989). Nevertheless, it is possible that other species of *Protuberata* may be immature stages of stinkhorns, especially given the range of relationships uncovered by Hosaka *et al.* (2006).

Most species of *Protuberata* (Malloch 1989) have the gleba divided into several discrete sections, and hence if they do turn out to be immature forms, they will be unrelated to *Ileodictyon*. There are, however, two species that have a single, central, gleba surrounded by a thick gelatinous layer which is traversed by radial threads (sometimes described as 'sutures'), and that also have well-developed rhizomorphs. These are *Protuberata africana* Lloyd from South Africa (Malloch 1989) and *P. parvispora* Castellano and R. Beever from New Zealand (Castellano and Beever 1994). In addition, *Rhizopogon rodwayi* McAlpine from Tasmania shares a very similar morphology, with a gelatinous peridium traversed by radial 'fibres' (McAlpine 1985). The illustration accompanying the type description of *Rhizopogon rodwayi* also shows a lattice-shaped pattern on the surface of the peridium. Despite the close morphological similarity of *P. parvispora* to *P. canescens*, DNA sequence data place the former species in the Protophallaceae, along with *P. maracuja* and several other species of *Protuberata* (Hosaka *et al.* 2006). Therefore, care must be taken in making judgements about relationships based solely on morphology.

The close similarity between *Protuberata canescens* and *P. africana* and *Rhizopogon rodwayi* suggests that these two species could well be immature *Ileodictyon*. It is also relevant that *Ileodictyon gracile* is known from Tasmania and South Africa (Cunningham 1944; Dring 1980), which is where *Protuberata africana* and *Rhizopogon rodwayi* occur. Cunningham (1944) already suggested that *Rhizopogon rodwayi* was a phalloid stinkhorn egg, and Chang and Kantvilas (1993) note that the type of the species has been annotated as being the young state of *Ileodictyon gracile* (as *Clathrus gracilis*). As with *Protuberata canescens*, until developmental differences between *I. gracile* and *I. cibarium* are known, it is not possible to definitely link *Protuberata africana* or *Rhizopogon rodwayi* to particular species of *Ileodictyon*.

#### Development in *Ileodictyon*

Connection of the two 'phases' of the Cage Fungus, *Protuberata canescens* and *Ileodictyon gracile*, indicates an unusual strategy for the timing of spore production in relation to fruit-body expansion.

In the fruit-bodies of epigeal fungi, such as agarics, mature spores are not present in un-

expanded fruit-bodies, but are produced once the primordial fruit-bodies have expanded significantly. In truffle-like fungi, where the fruit-body remains wholly or partially buried, the fruit-body never expands and mature spores remain within the un-ruptured peridium. Dispersal of truffle-like fungi is usually the result of ingestion by mammals. In contrast, the Cage Fungus has an enclosed, truffle-like fruit-body, initially in which to produce spores, with the benefit of protection from desiccation, but then switches to an epigeal fruit-body, which gives access to insects (such as flies) which are presumed to disperse the spores. Insects are attracted to the foetid-smelling spore masses adhering to the inner surface of the lattice, which explodes to its full size on rupturing of the peridium (Bougher and Syme 1998).

The Altona North material was initially half-buried and the '*Protuberata canescens*' collection from Belgrave South was hypogaeal. Bougher and Malajczuk (1986) note that Western Australian material of *Protuberata canescens* was mostly hypogaeal and decayed *in situ*. Further observations are required on the proportion of fruit-bodies that remain buried and unexpanded, and whether this is due to unfavourable weather conditions or other factors. The role of insect larvae observed to devour the gleba in unopened fruit-bodies also needs investigation as to whether this provides the fungus with an alternative method of spore dispersal.

Initially, in the '*Protuberata*' stage, we observed a lattice 'in miniature' surrounding the relatively firm gleba. However, just before expanding, the lattice occupies the whole interior of the fruit-body, with the arms tightly folded within the peridium (Semmens 1967). Growth in the length of the lattice arms apparently pushes the lattice into the area initially occupied by the gleba. The gelatinous middle layer of the peridium becomes a little thinner before the peridium ruptures (Semmens 1967), which would also create some extra room for expansion of the lattice. Thus, production of spores within the unopened Cage Fungus fruit-body forms a slimy mass which adheres to the arms of the lattice as it expands before bursting from the peridium. Presumably, spores are mature before the lattice bursts from within the peridium.

Comparison of spore dimensions for *Ileodictyon gracile* and the various *Protuberata* and *Rhizopogon* discussed above shows only minor

**Table 1.** Spore dimensions reported in the literature for species of *Ileodictyon* and selected species of *Protuberata*.

Species	Spore length × width (µm)	Reference
<i>Ileodictyon gracile</i>	5–6.5(–7.5) × 2–2.5	Bougher and Syme (1998)
<i>Ileodictyon gracile</i>	4.5–6 × 1.5–2.5	Cunningham (1944)
<i>Ileodictyon cibarium</i>	4–6 × 1.8–2.5	Cunningham (1944)
<i>Protuberata canescens</i>	4–5.5 × 2–2.5	Beaton and Malajczuk (1986)
<i>Protuberata canescens</i>	4–5(–5.5) × 2–2.5	Bougher and Syme (1998)
<i>Protuberata africana</i>	4.6–6.2 × 2–2.8	Malloch (1989)

differences (Table 1), which could well be due to spores in the gleba of unopened fruit-bodies not being fully mature (such as at a stage when the lattice arms were at a very early stage of development). Further comparison of spore dimensions in the sequence from unopened to fully open fruit-bodies would be of interest, as would tests of maturity (such as whether spores can germinate).

### Conclusion

*Protuberata canescens* is considered to be an early developmental stage of *Ileodictyon*, most likely *I. gracile*. Consequently, the name *Protuberata canescens* should only be applied in terms of it being a stage of *Ileodictyon*. Unexpanded fruit-bodies of *Ileodictyon* should be observed to see what is the shape of the lattice that eventually emerges, in order to identify the particular species. Fresh collections of *Ileodictyon cibarium* need to be examined carefully to see what is the form of the unexpanded fruit-body, and how it might differ from that of *Ileodictyon gracile*.

The original descriptions of *Ileodictyon gracile* and *I. cibarium* both date from the 1840s and hence, if indeed any or all of *Protuberata canescens*, *P. africana* and *Rhizopogon rodwayi* are confirmed as synonyms of particular species, they do not threaten the priority of the two long-established names in *Ileodictyon*.

### Specimens Examined

Material of *Ileodictyon gracile* (including the *P. canescens* stage) from Altona North has been lodged at the National Herbarium of Victoria (MEL) and the Herbarium, Royal Botanic Gardens Kew (K) (N. Sinnott 3535, 3548, 3566, 3631 and 3642). The collection from Belgrave South is lodged at MEL (H. Weatherhead s.n., =T.W. May B534).

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